

Figure 1.

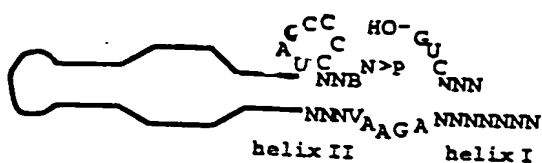
10067956-020502

Trans Cleavage and Ligation

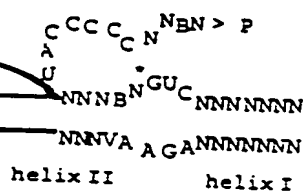
T7 promoter

Library Sequences

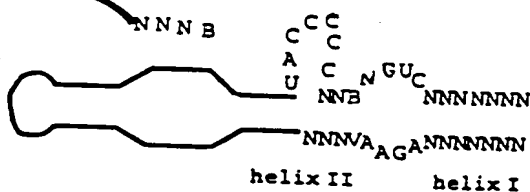
- Transcribe library with T7 RNA polymerase.
- Cis-cleavage occurs.
- Purify those randomized ribozymes that have undergone cis-cleavage.



React with target RNA in Trans



Trans Ligation

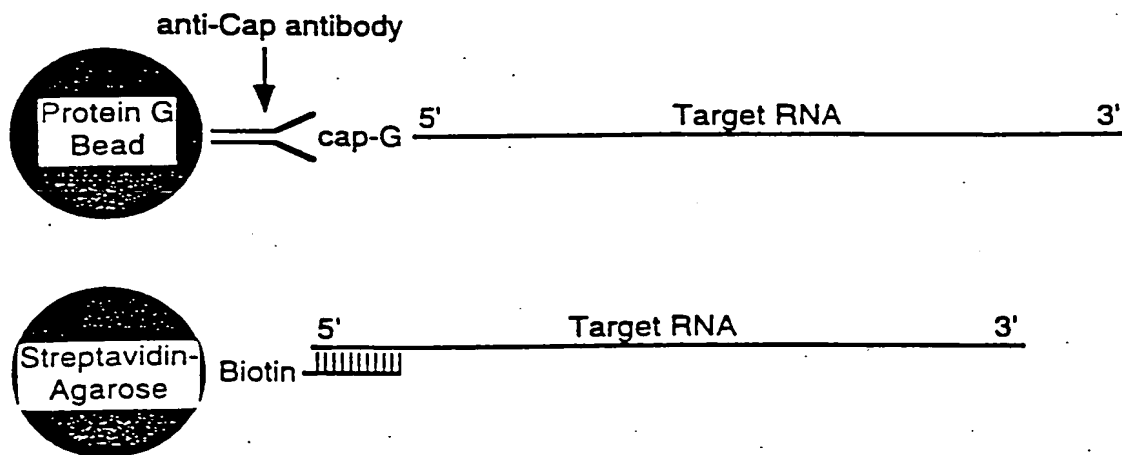


- Trans-ligated products are isolated and amplified by RT-PCR.
- Trans-ligated ribozymes can then be further amplified and subcloned into AAV vectors for production of a target specific ribozyme gene vector library.

Fig. 2

Attaching RNA target to solid support

Binding target RNA at 5' end:



Binding target RNA at 3' end:

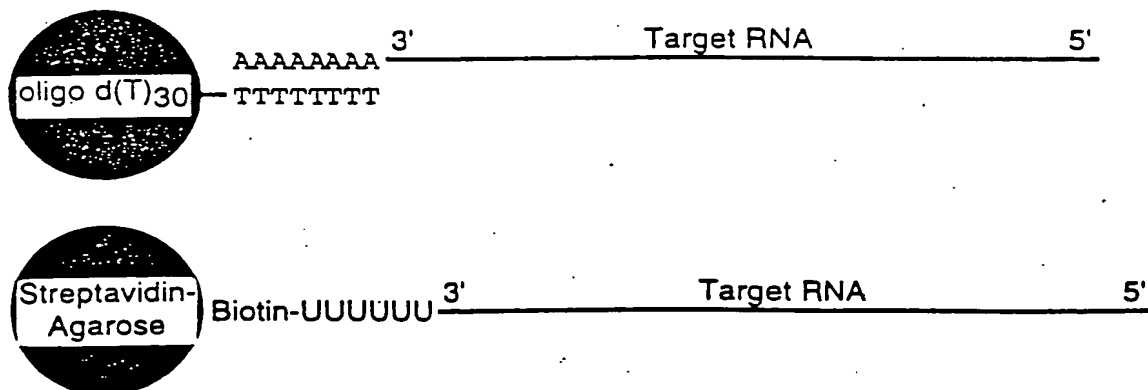
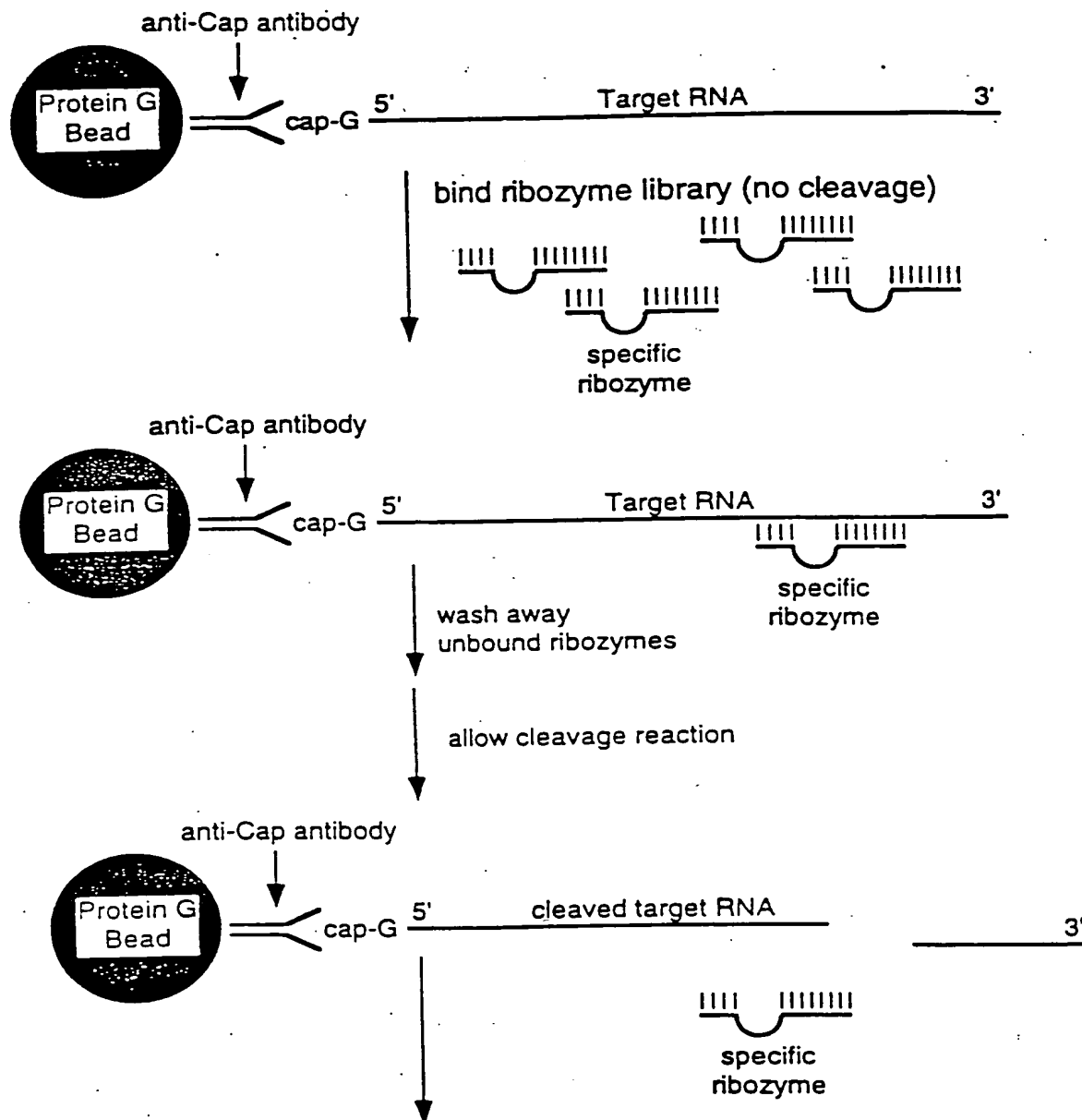


Fig. 3

In vitro selection of optimal ribozymes



Specific ribozymes elute off the solid support
 Amplify, synthesize and re-apply ribozymes to new column
 Carry out selection multiple times, as necessary

Fig. 4

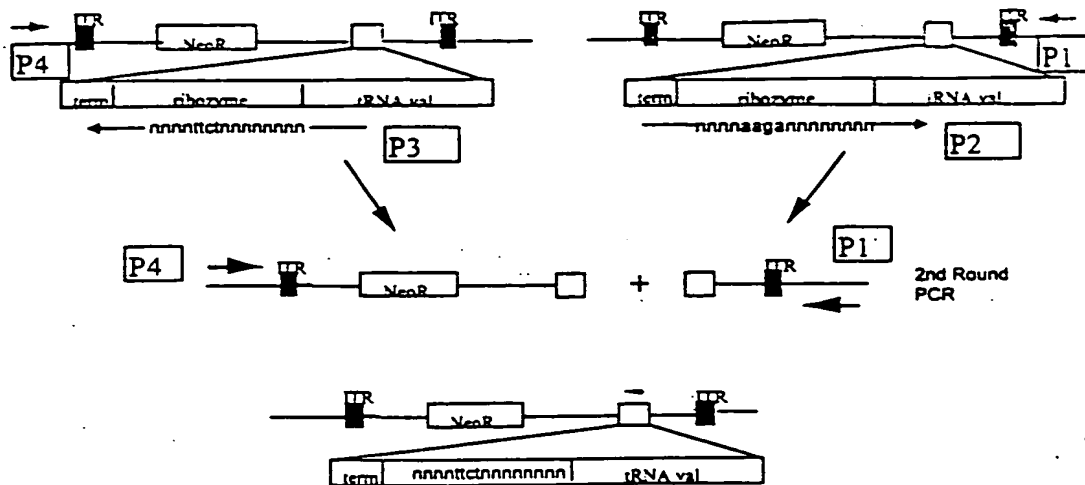


Fig. 5

205020-95679001

Cleavage of Various Target RNAs with AAV-Ribozyme Vector Library

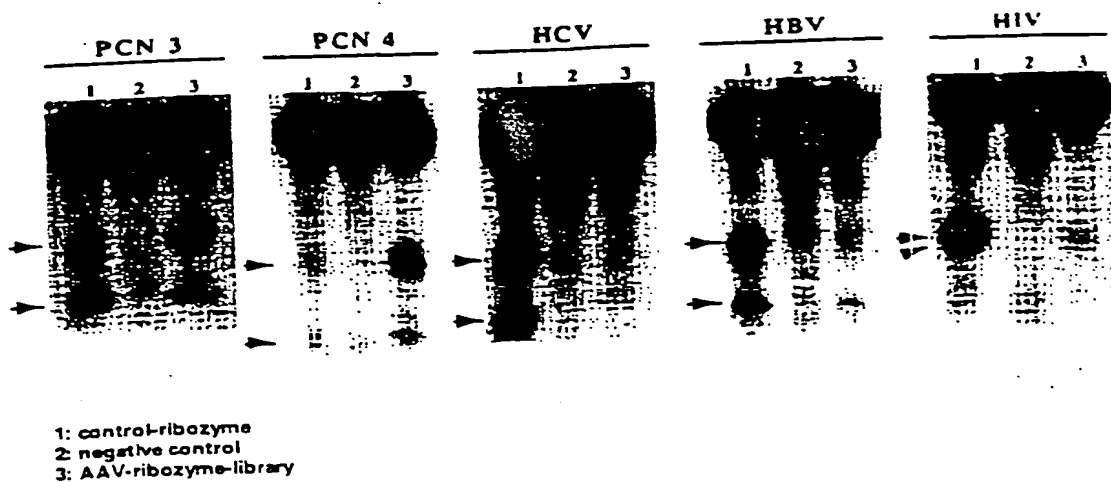


Fig. 6

205020-9556-020502

p1014/2kb

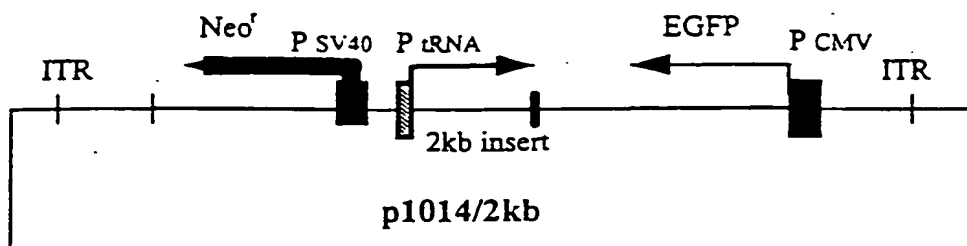


Fig. 7

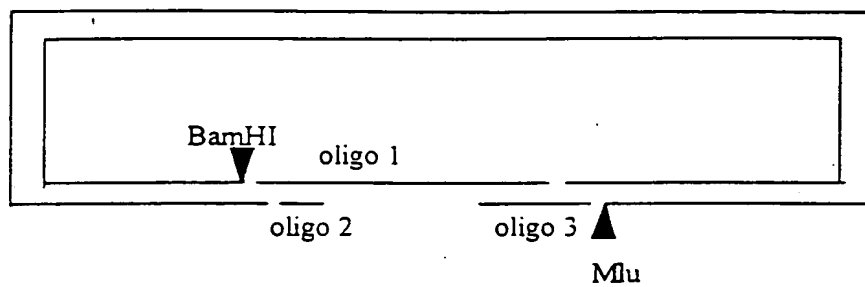


Fig. 8

205020-956/9001

pAAV-Hygro-PGK

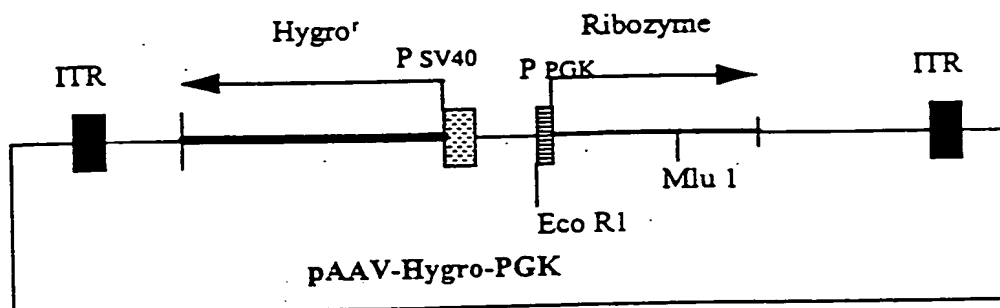


Fig. 9

pPol II/PGK_{mus}/neo

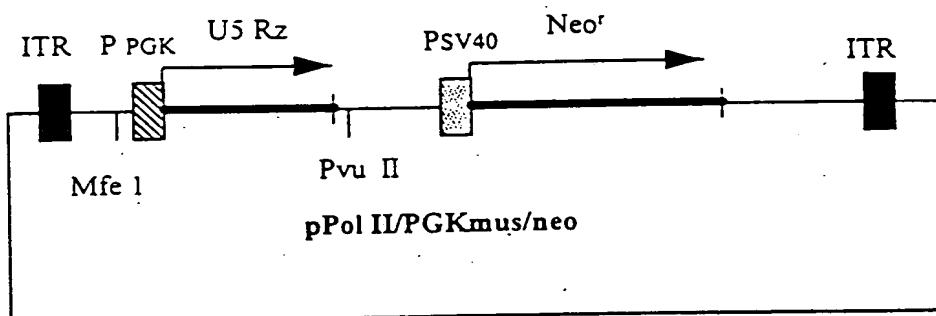


Fig. 10

205020" 956/900T

p 1015

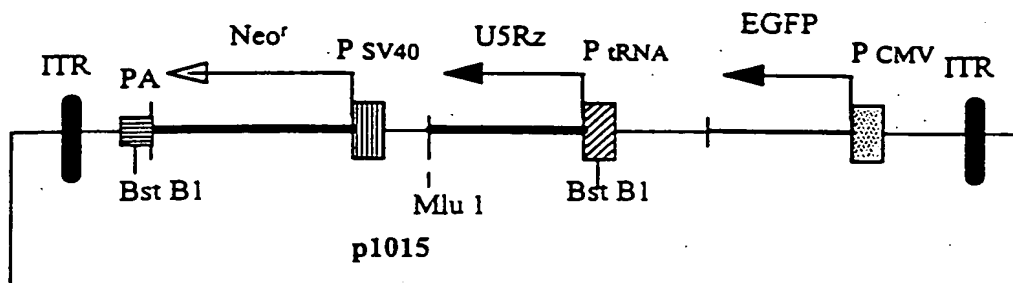
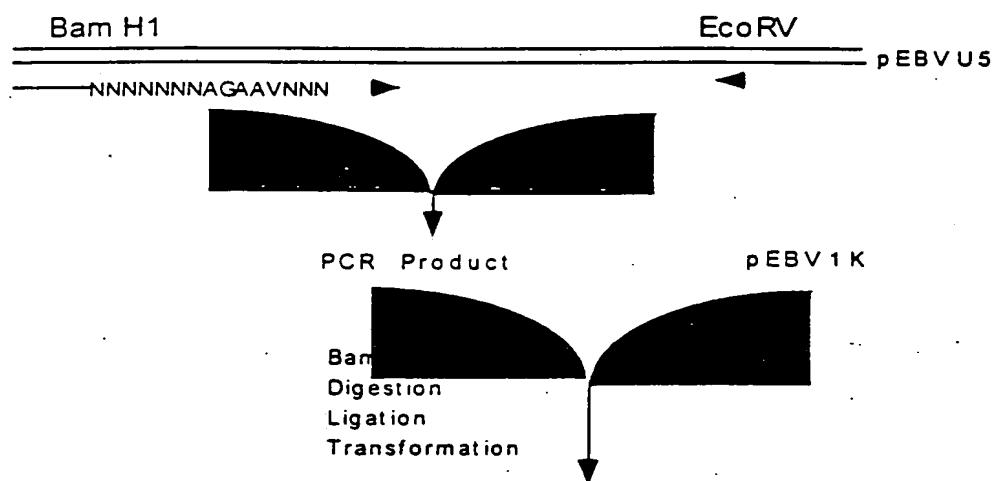


Fig. 11



ERL030398

Fig. 12

10067956-020502

pLHPM-2kb

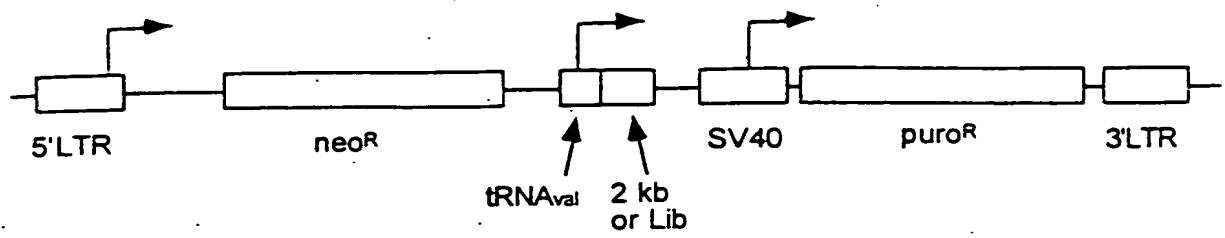


Fig. 13a

pLPR-2kb

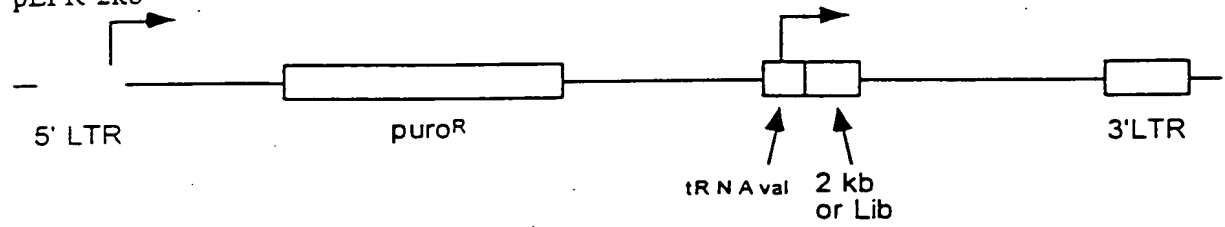


Fig. 13b

205020-956/9004

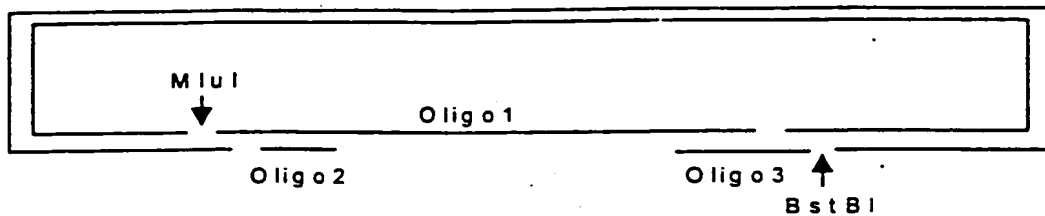


Fig. 14

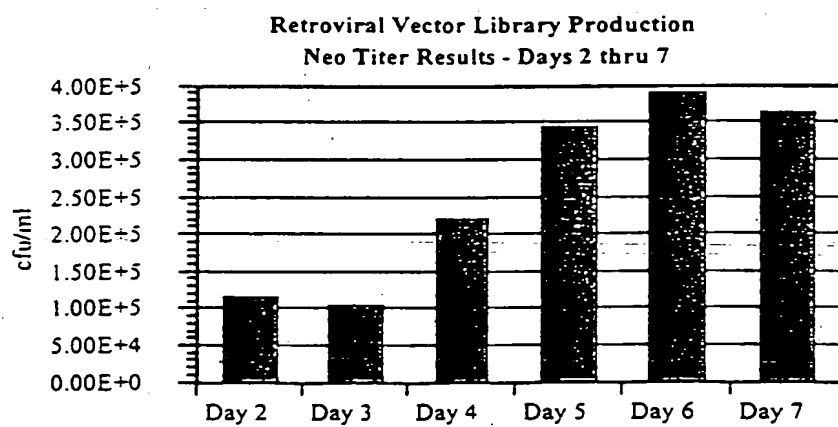


Fig. 15

205020-95679001

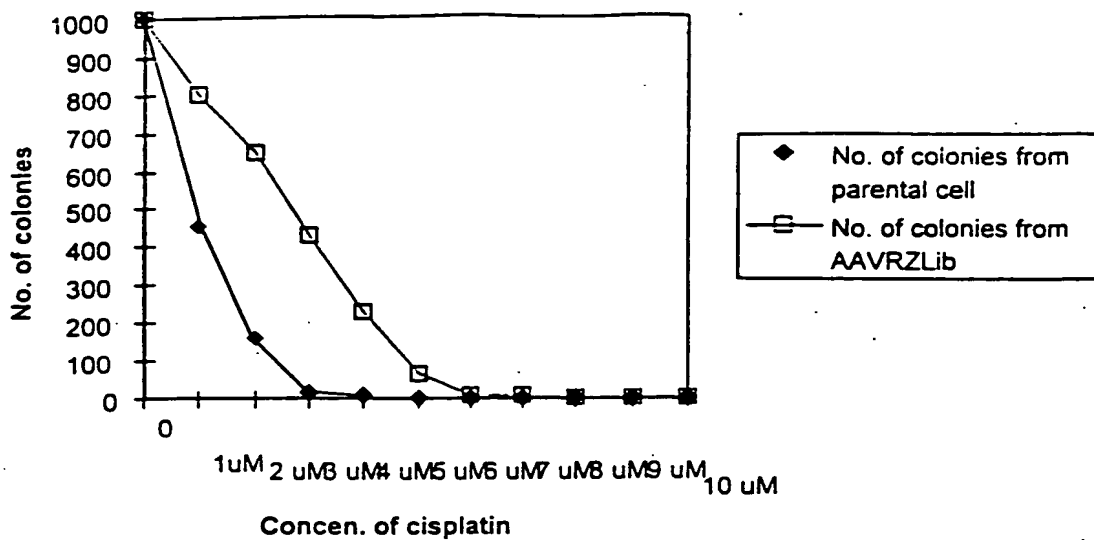


Fig. 16

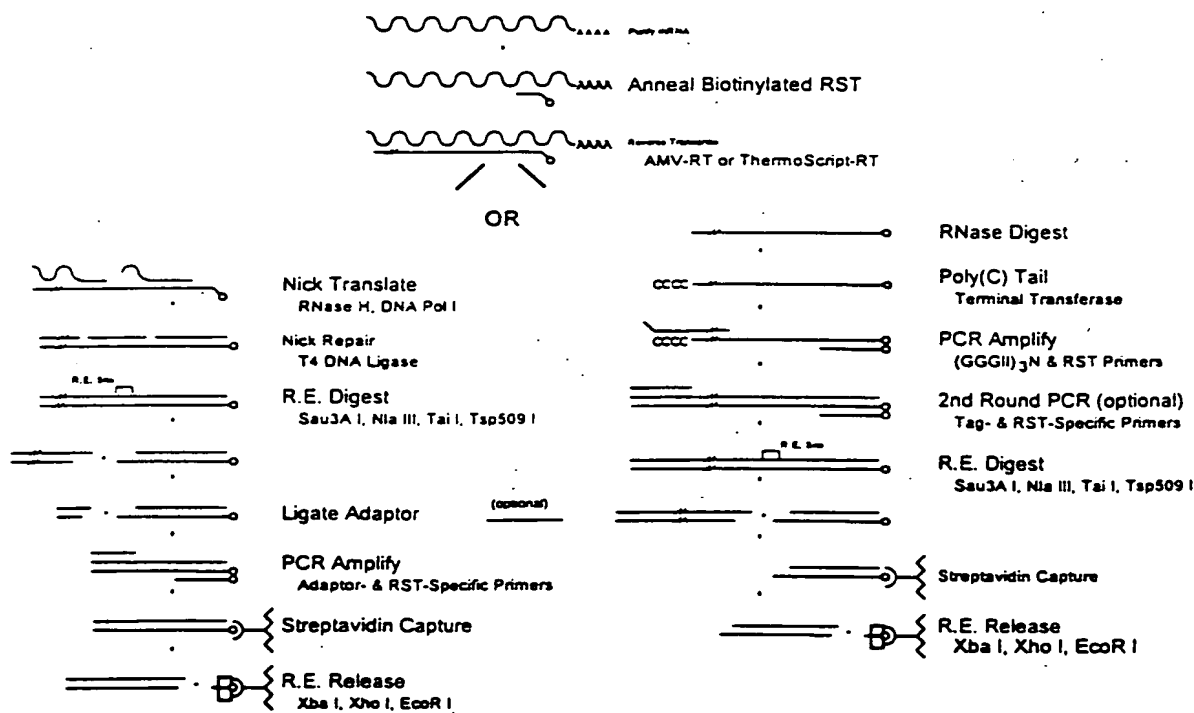


Fig. 17

205020-956/9001

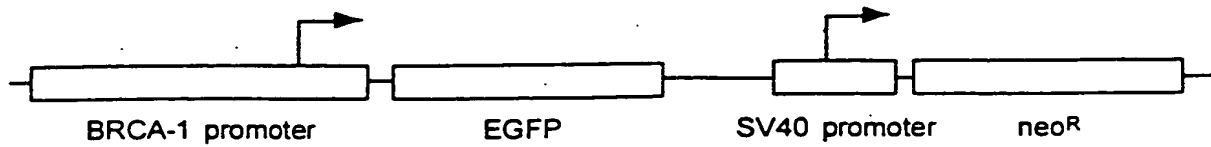


Fig. 18

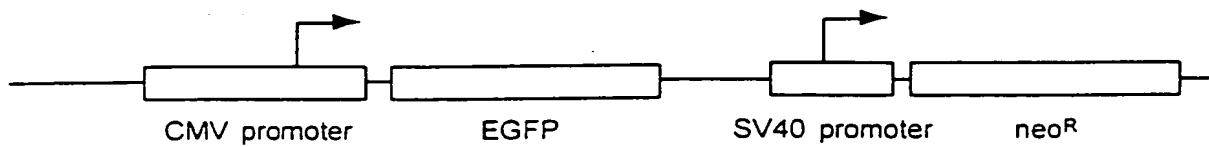


Fig. 19

20050209 095629004

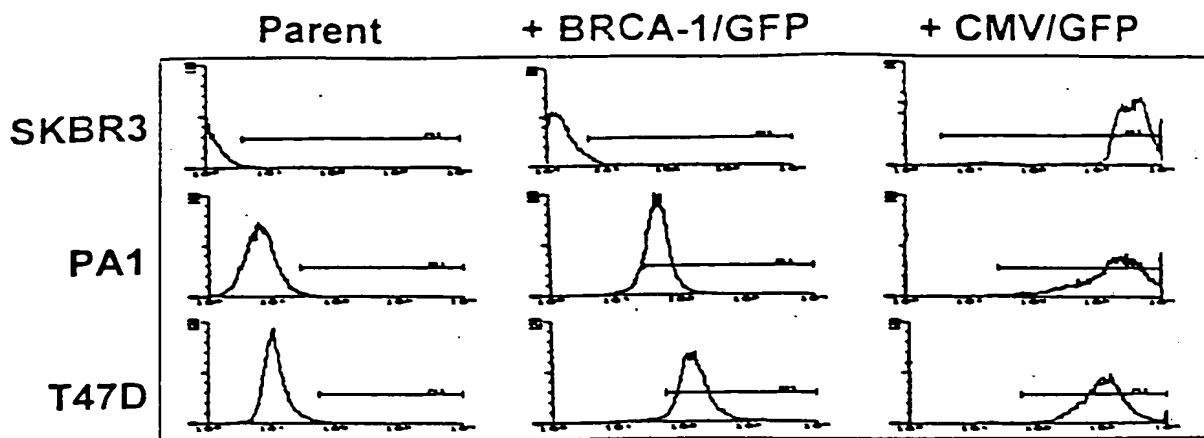


Fig. 20

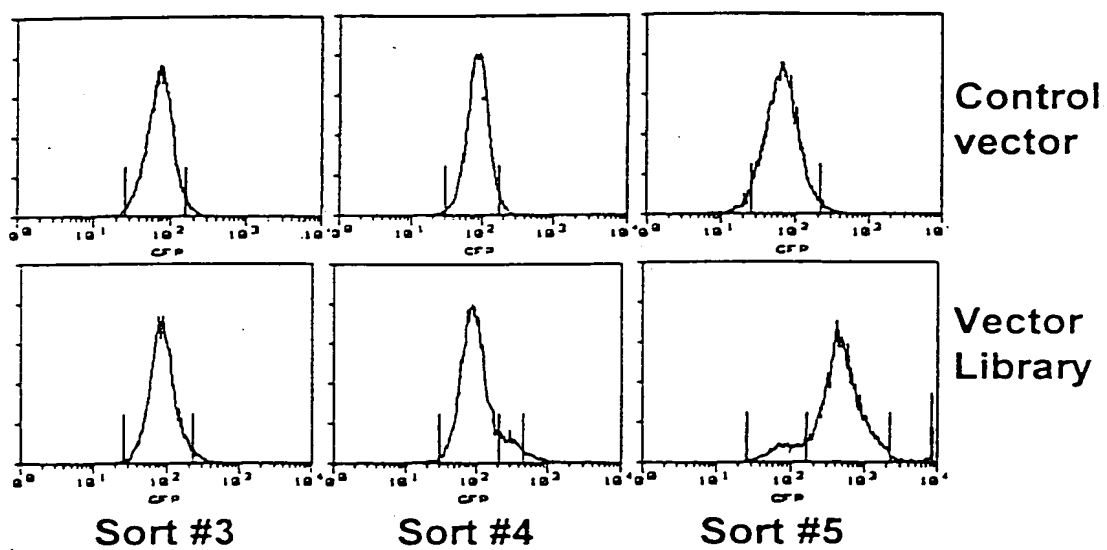


Fig. 21

205020-956/9001

SV 40 - hygro - HCV IRES - HSV-tk

Fig. 22

Transfection (Electroporation)

HeLa

hygromycin
selection

stable cell line
SV40-hygro-IRES-tk (5' TK)

no ribozyme

no inhibition of
IRES translation

HSV-tk expression

cell death

RzLibrary

inhibition of
IRES translation

no HSV-tk

cell survival

Ganciclovir

Fig. 23

205020-956/9007

Figure 24

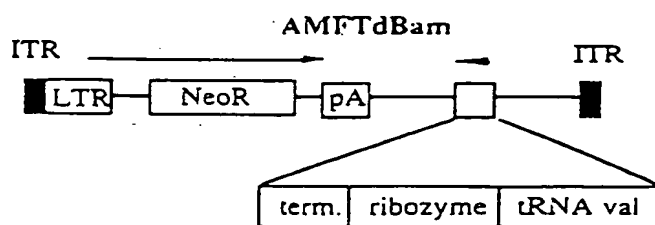
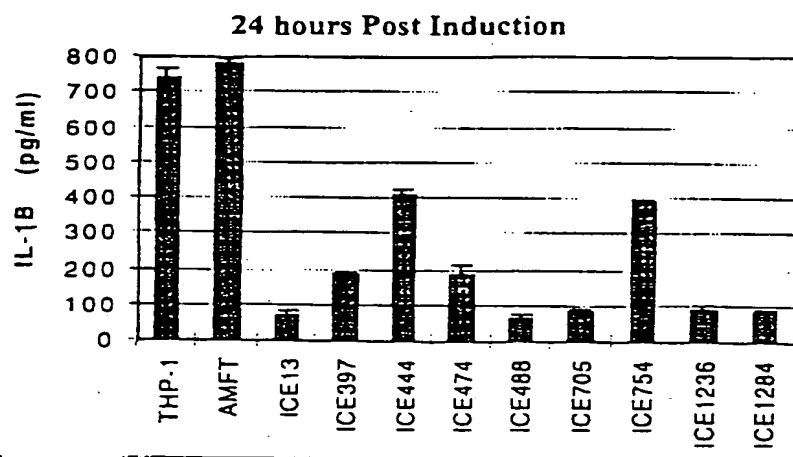
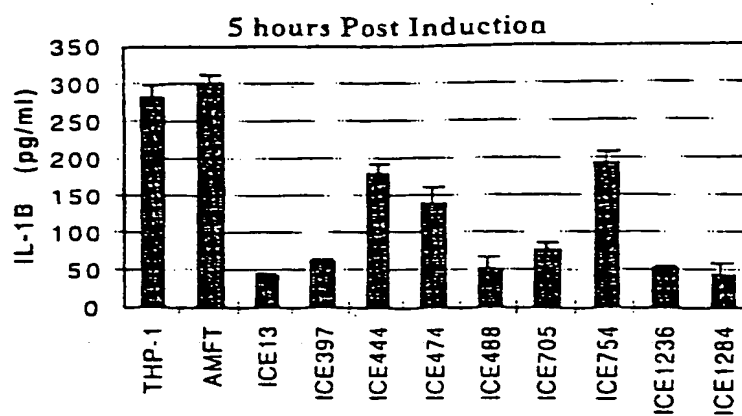


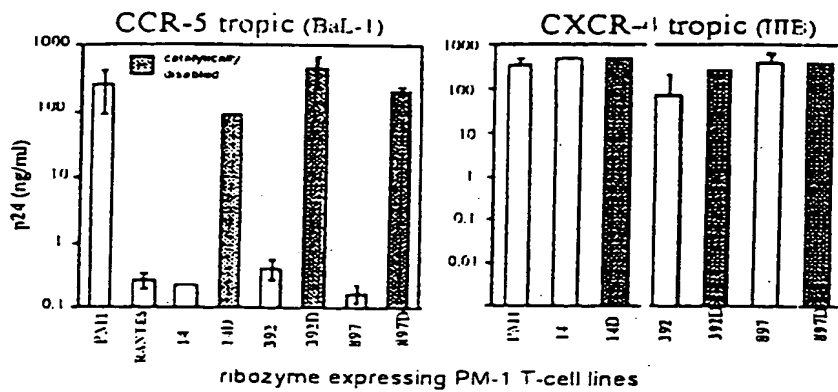
Figure 25



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Figure 26

Resistance to CCR-5 tropic HIV



Surface expression

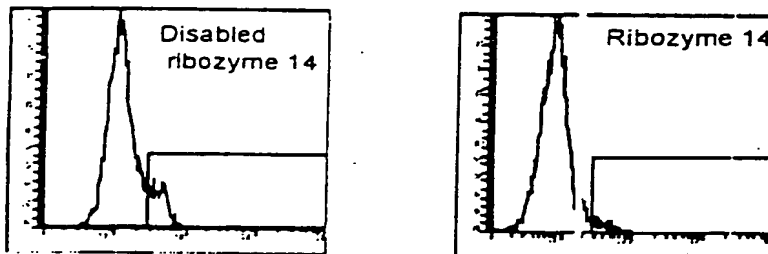
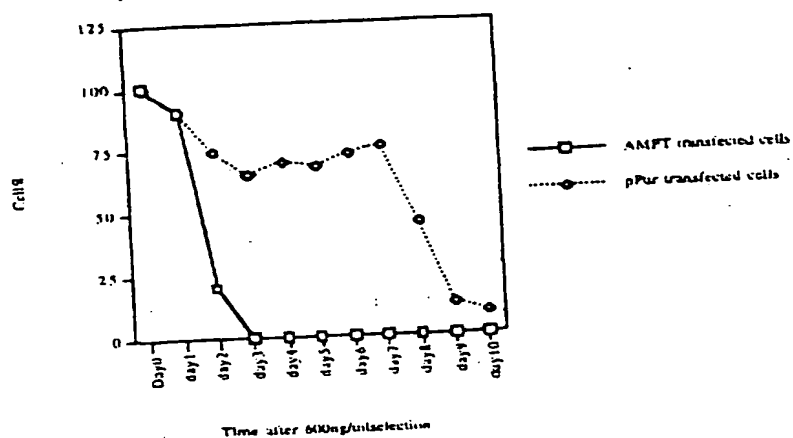


Figure 27

Puromycin selection on pPur and AMFT transfected A549 cells



Puromycin selection on pPur and AMFT transfected Hela cells

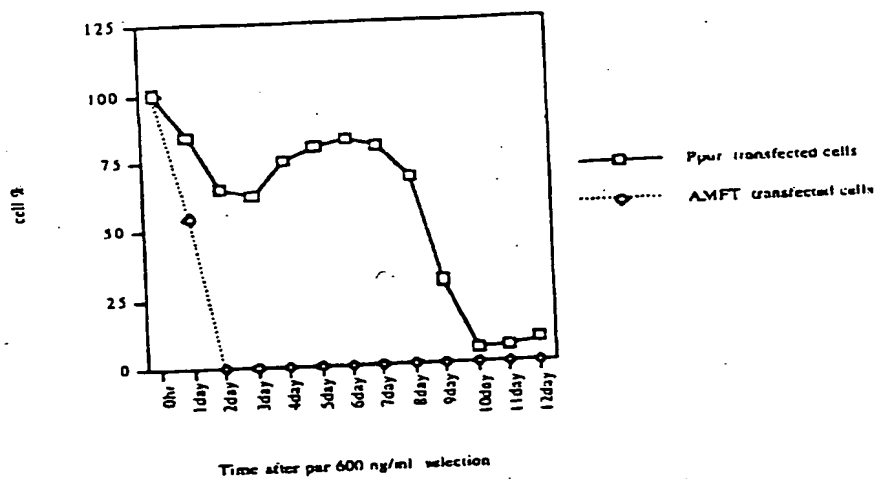
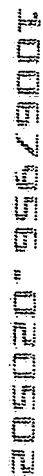


Figure 28



205020 956/900T

Figure 29

Auxiliary Sequences

Time Course for Cleavage by U5 - SLII Oligomeric Ribozymes

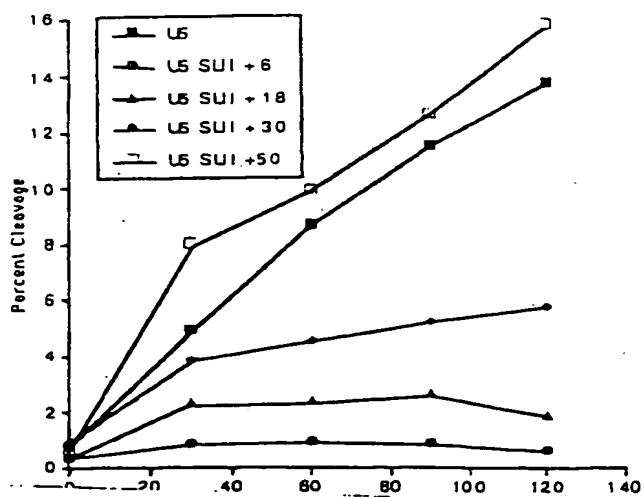
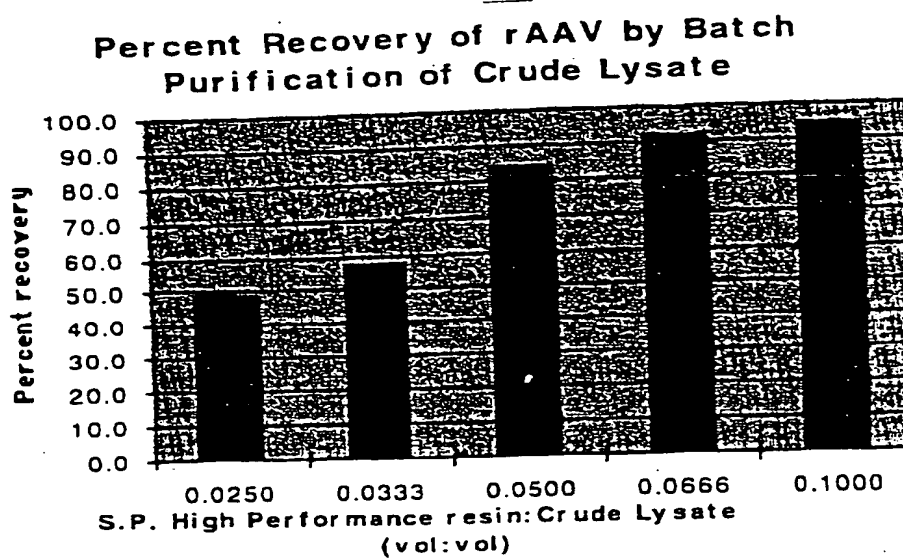


Figure 30



205020-95629001